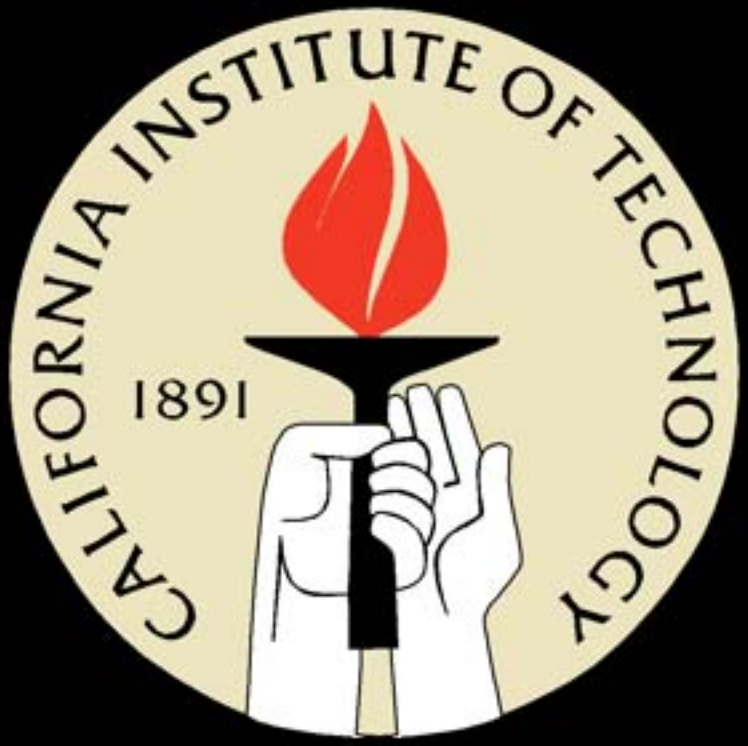
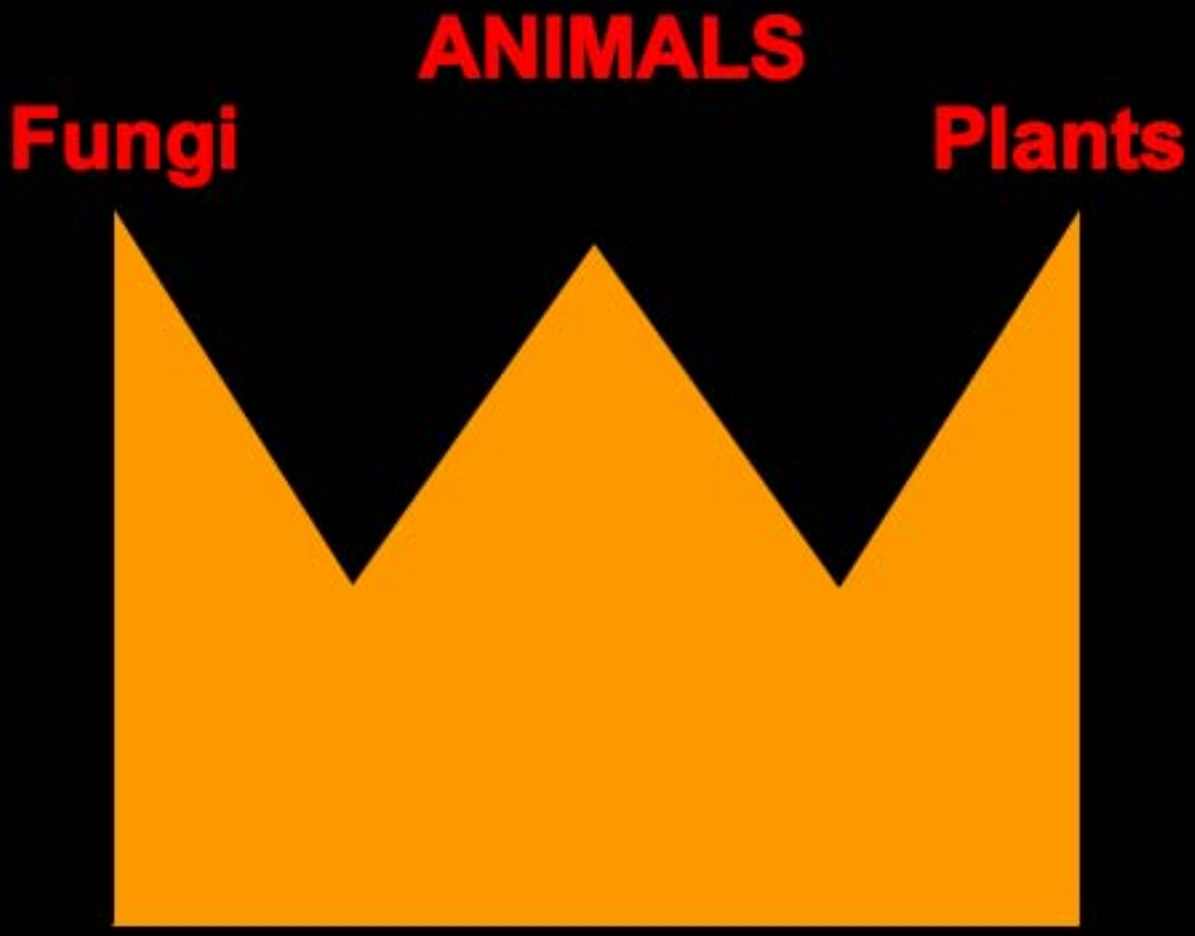

CALIFORNIA INSTITUTE OF TECHNOLOGY
1891

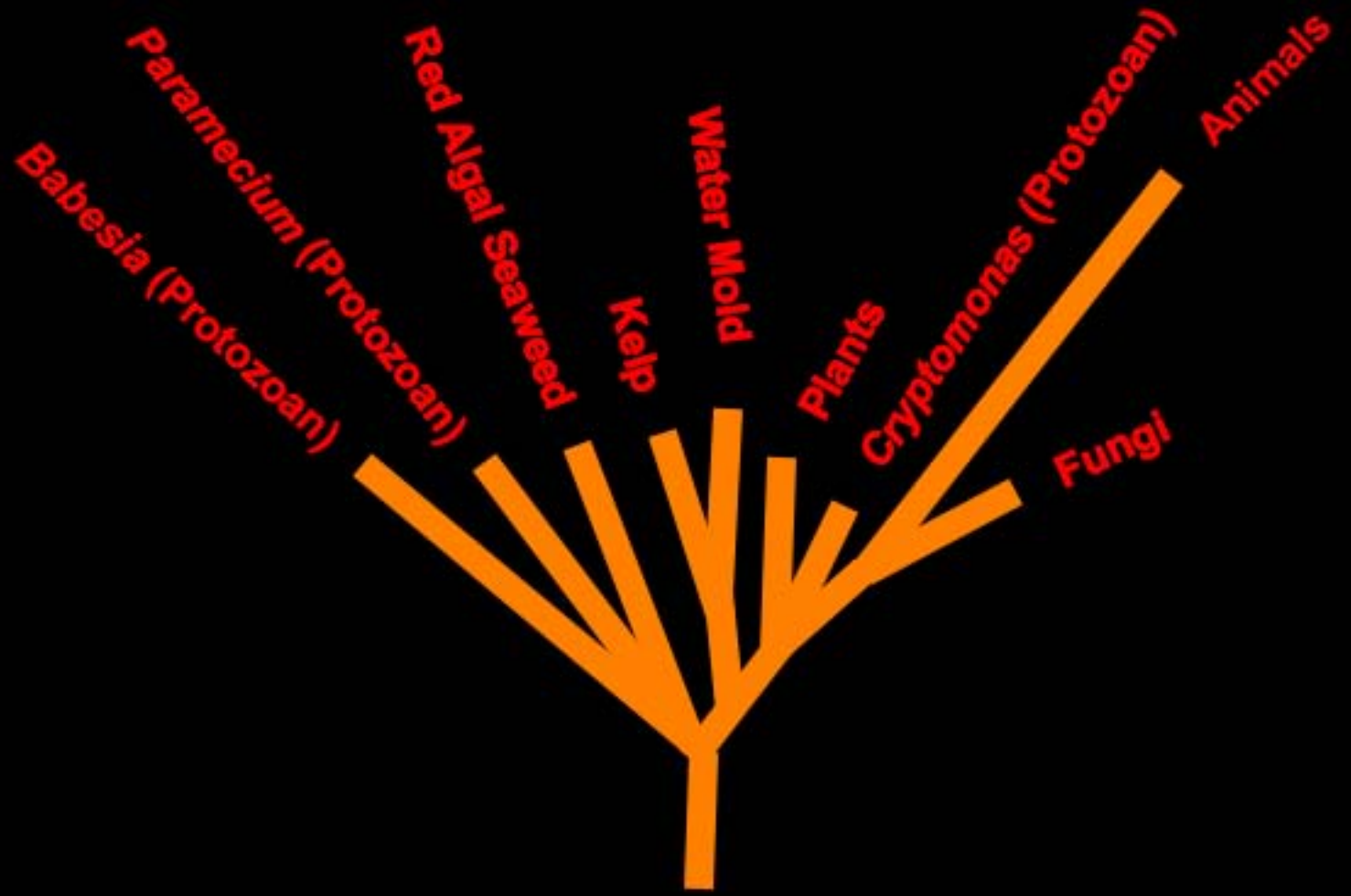
OVERVIEW

- Introduction to biological diversity, wood, termites, and termite hindgut microbiology
- Collecting and sampling in Costa Rica
- Metagenomic statistics
- Vignettes: Cellulose, Xylan, and Hydrogen



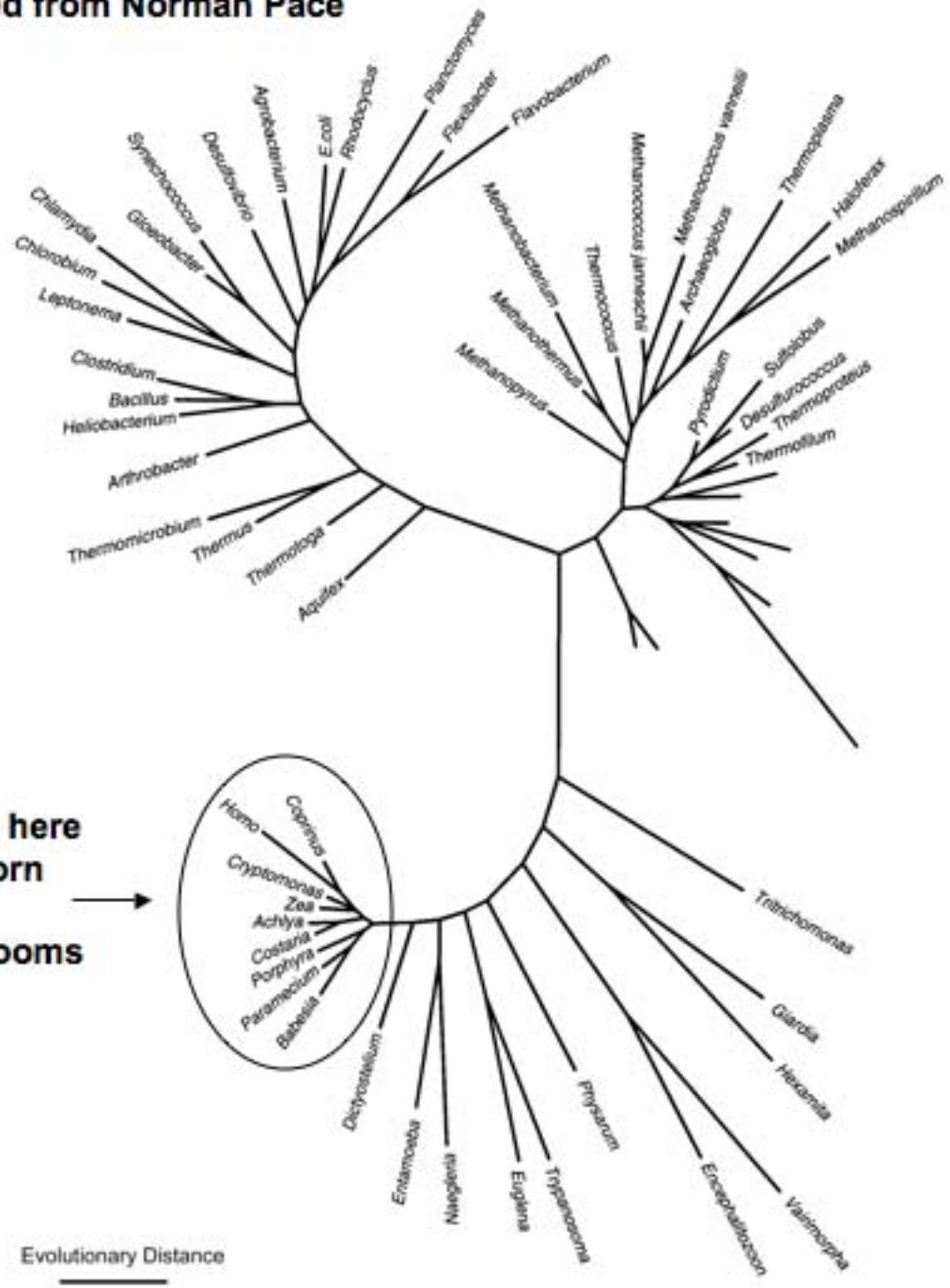
The Crown Taxa or…..

….the emperor has no clothes?



Adapted from Pace and coworkers

Adapted from Norman Pace



What is lignocellulose?

- Most abundant form of biomass globally
- Nature's first composite material
- Complex tri-laminate of three distinct polymers: Cellulose, Xylan, and Lignin
- Structure in native-form is poorly understood
- Degradability of different preparations of purified homopolymers varies widely
- Xylan & lignin degradation studies typically use chemically denatured substrate

What is the challenge to converting or degrading lignocellulose into anything?

Should the focus be on, as examples: "*Ethanol Ethanol Ethanol*", or "*Pine into Pyruvate*"?

Modularity in metabolism

Modularity in metabolism

Sucrose (disaccharide)

Hexose (6 Carbon Sugar)
Pentose (5 Carbon Sugar)

Modularity in metabolism

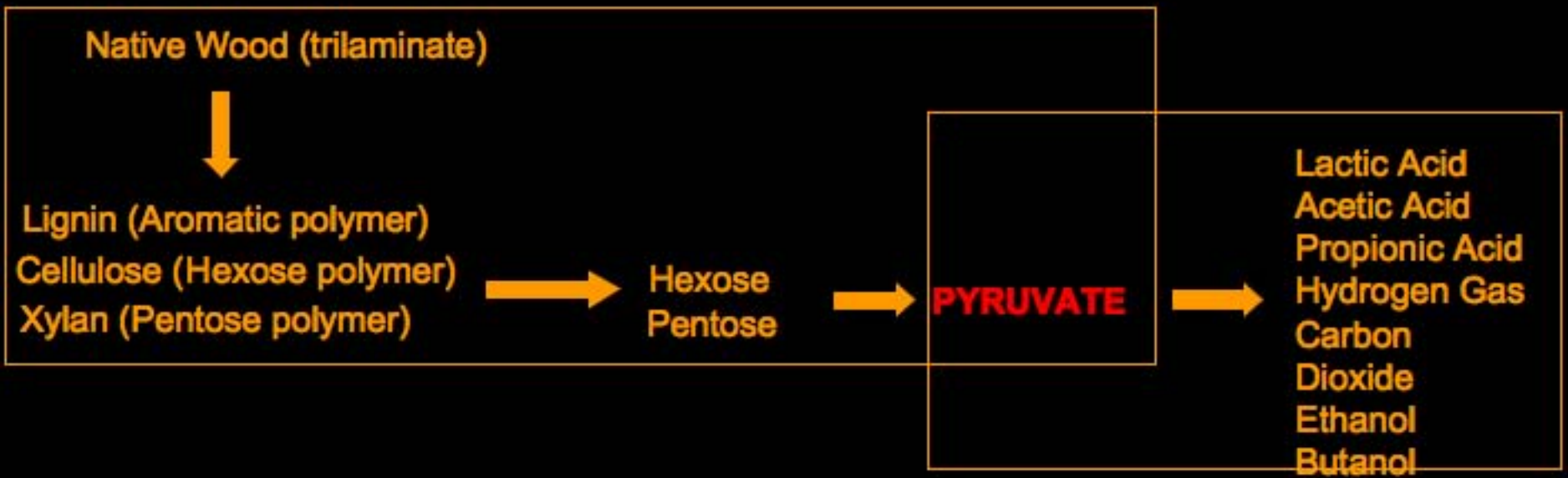
Sucrose (disaccharide)

Hexose (6 Carbon Sugar)
Pentose (5 Carbon Sugar)

PYRUVATE

Lactic Acid
Acetic Acid
Propionic Acid
Hydrogen Gas
Carbon Dioxide
Ethanol
Butanol

Modularity in metabolism



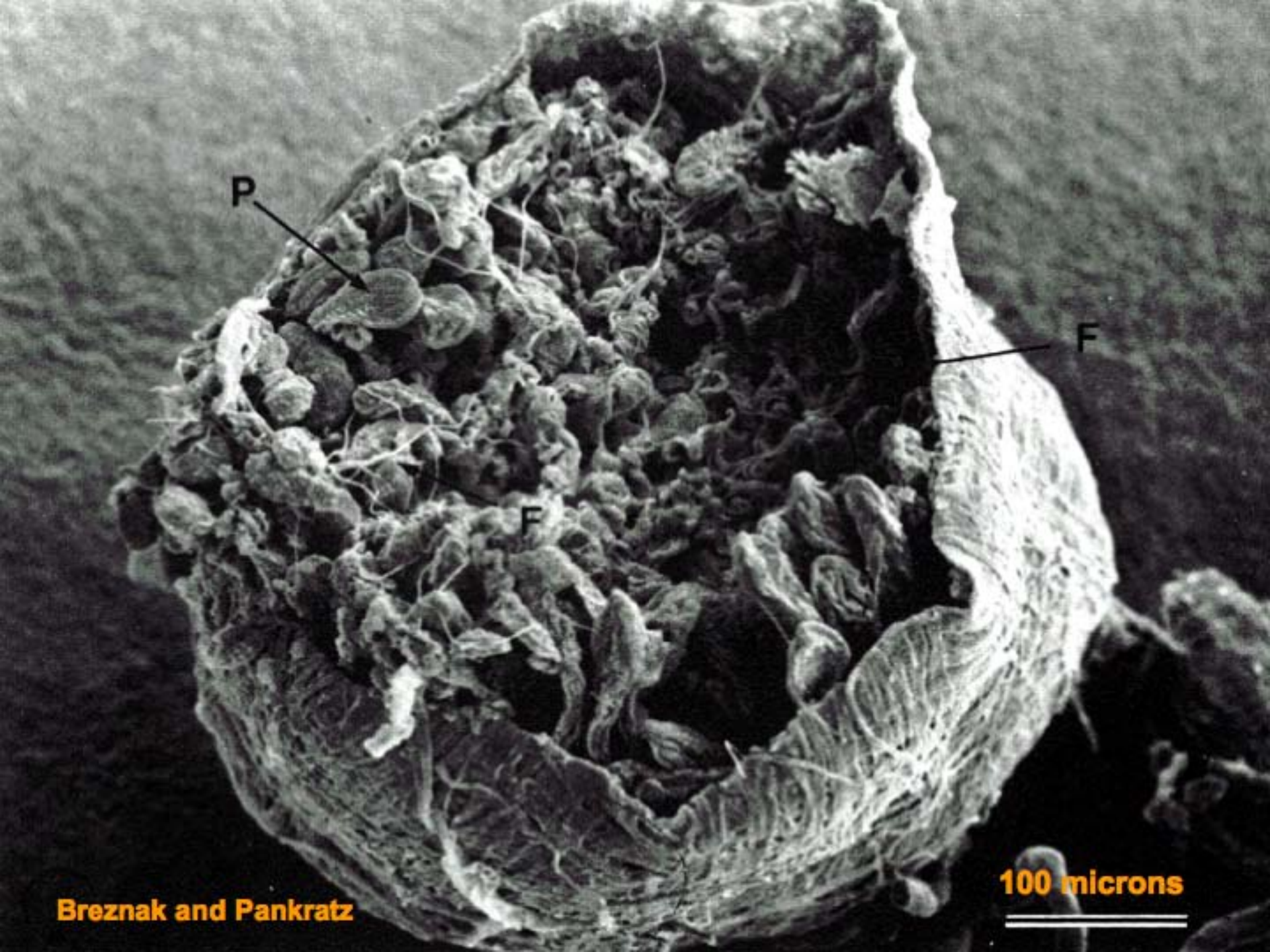
There is a critical basic and applied need to study and understand natural systems that already degrade and convert native lignocellulose into…… pyruvate.

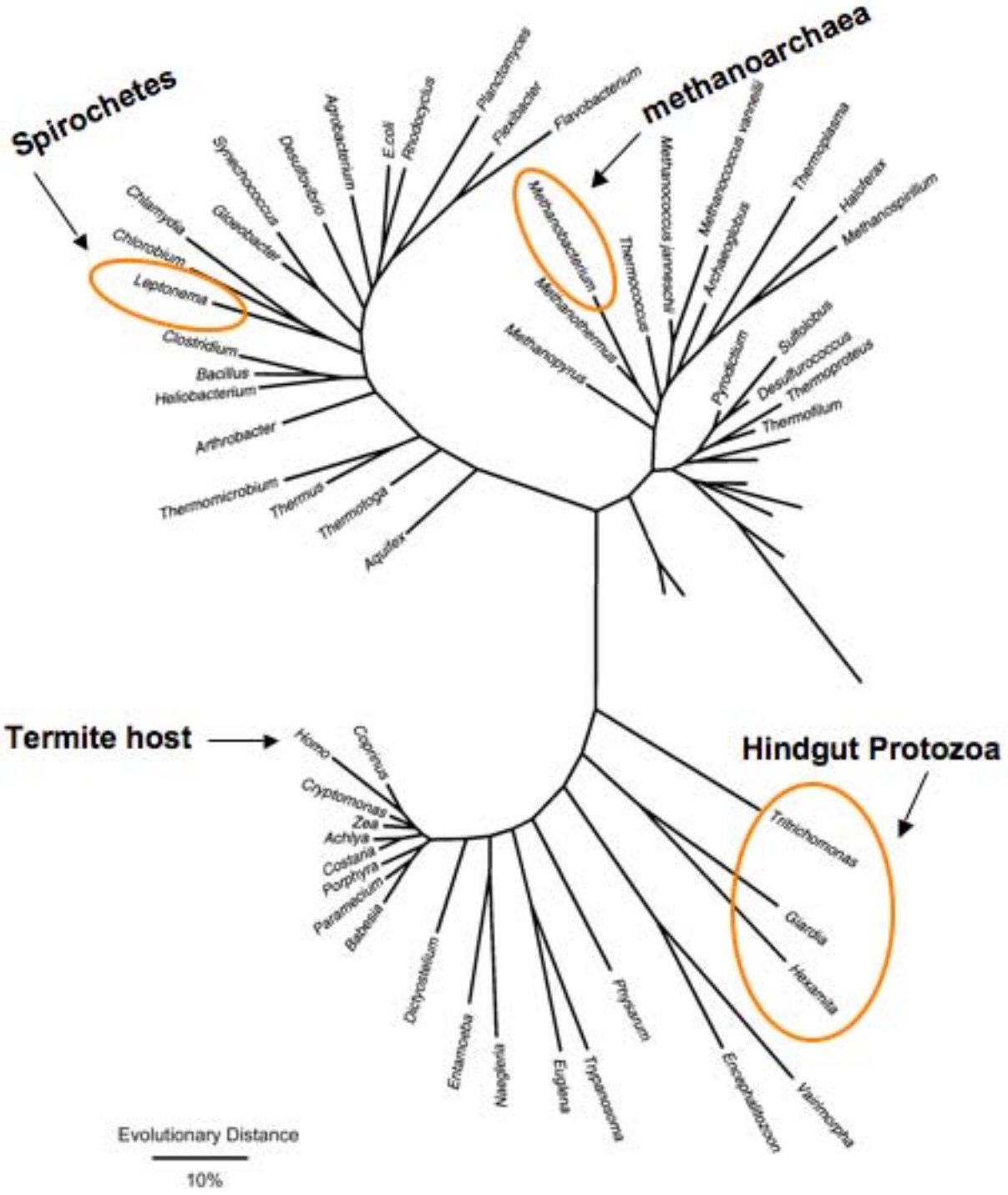
Once you get there, there are many options that can be explored.

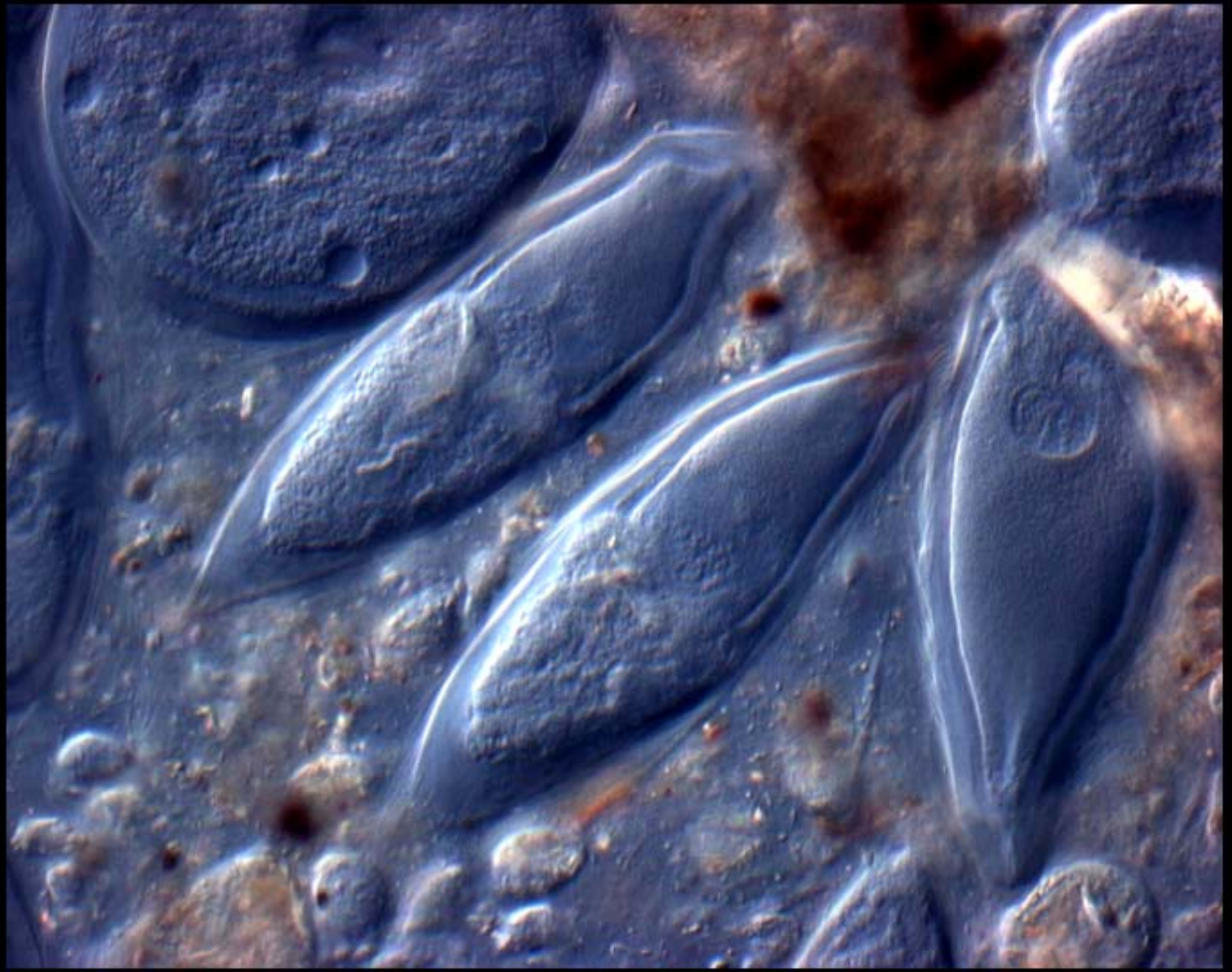
Ponderosa at ca. 6,000-7000 ft on Mt. Pinos

The Dampwood Termite *Zootermopsis nevadensis*

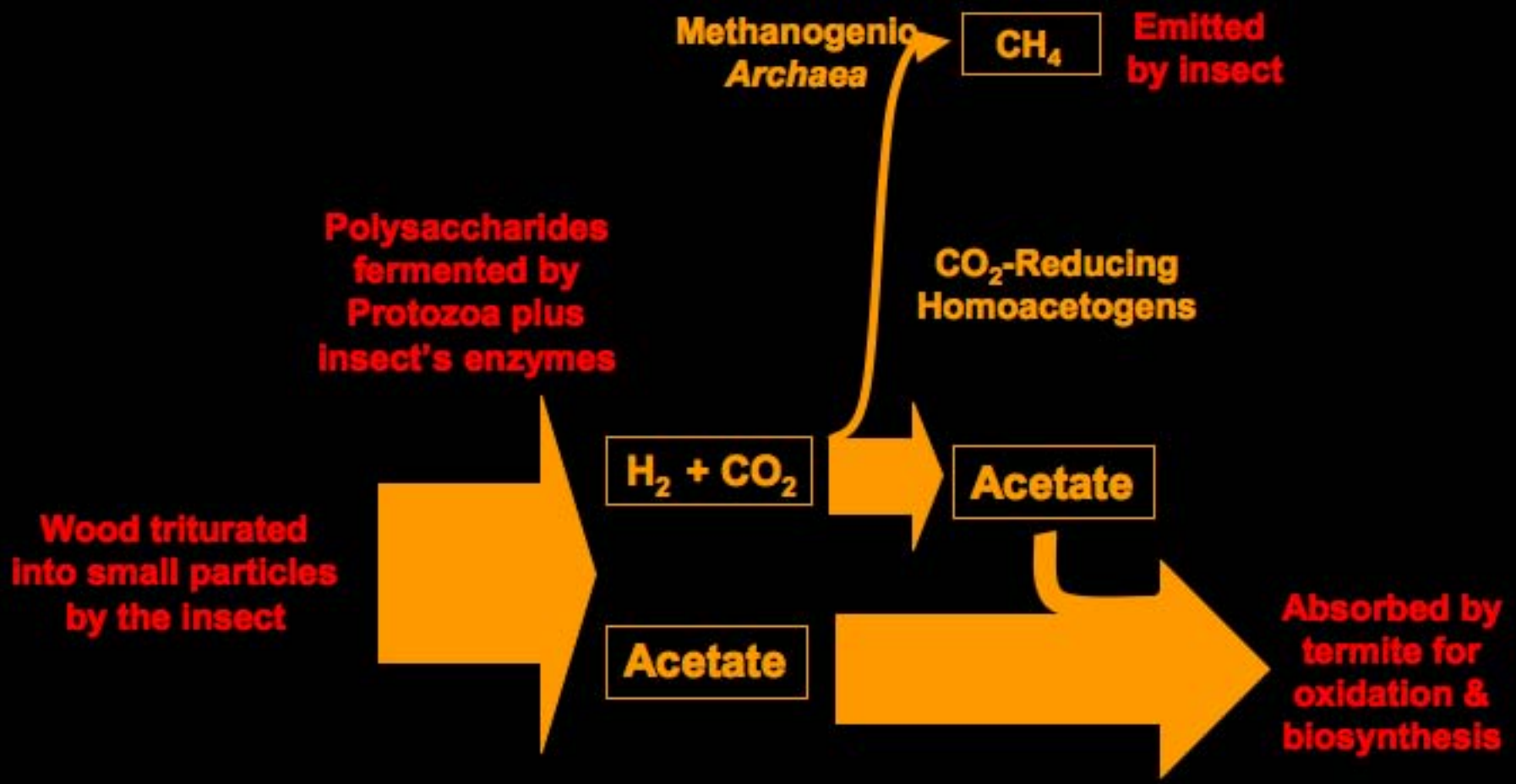
Salmassi and Leadbetter


P
F
F
100 microns
Breznak and Pankratz

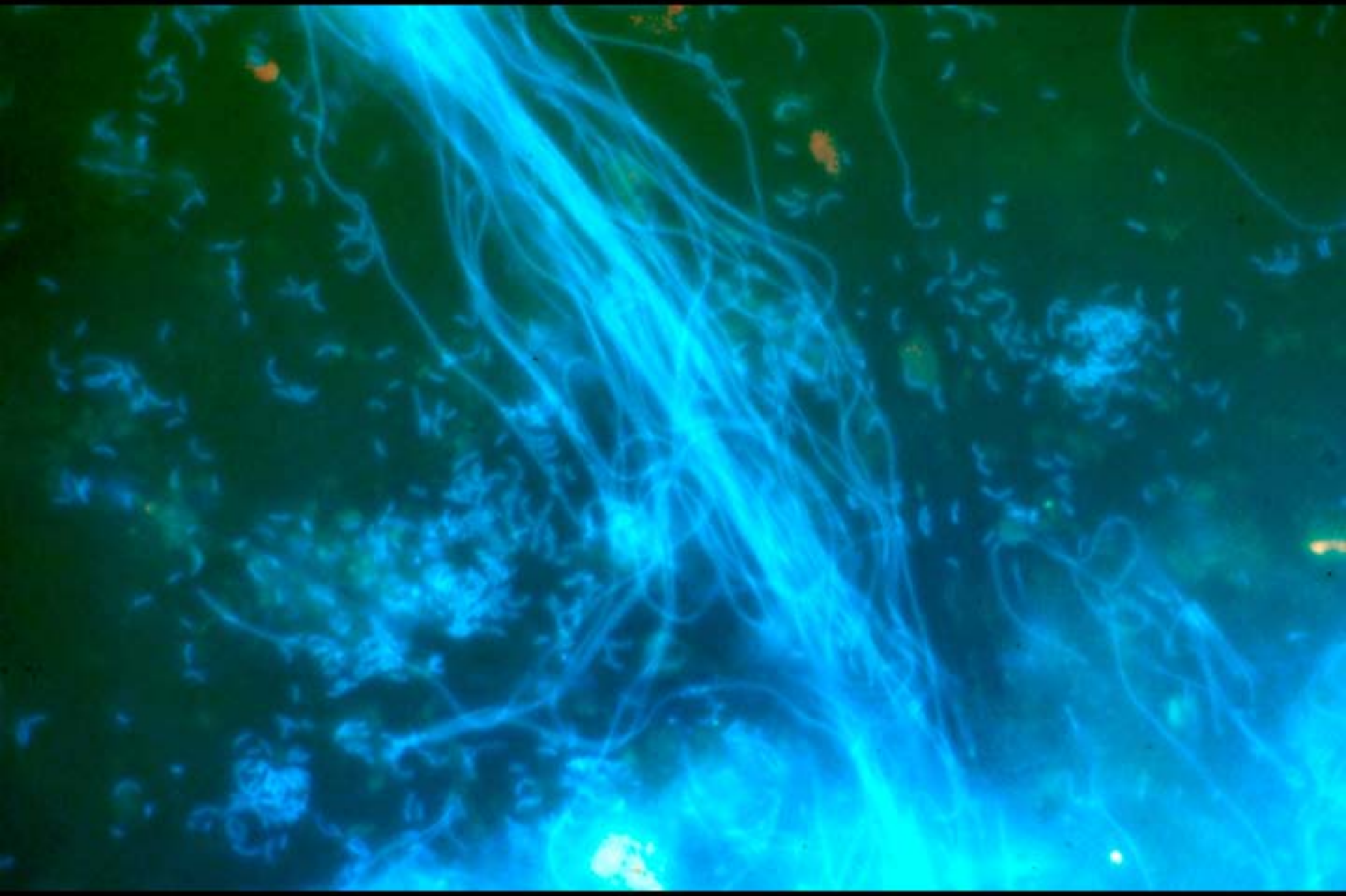

Spirochetes
methanoarchaea
Termite host
Hindgut Protozoa
Evolutionary Distance
10%
Flavobacterium
Planctomyces
Flexibacter
E.coli
Rhodocyclus
Agrobacterium
Desulfovibrio
Synechococcus
Gloeobacter
Chlamydia
Chlorobium
Leptonema
Clostridium
Bacillus
Helicobacterium
Arthrobacter
Thermomicrobium
Thermus
Thermodoga
Aquifex
Methanobacterium
Methanothermus
Methanopyrus
Thermococcus
Methanococcus jannaschii
Methanococcus vannielii
Archaeoglobus
Thermoplasma
Halobacterium
Methanospirillum
Pyrodictium
Sulfolobus
Desulfurococcus
Thermoproteus
Thermofilum
Homo
Canis
Cryptomonas
Zea
Achlya
Costaria
Porphyra
Paramecium
Babesia
Dictyostelium
Entamoeba
Naegleria
Euglena
Trypanosoma
Physarum
Encephalitozoon
Vairimorpha
Trichomonas
Giardia
Hexamita



GENERAL SCHEME UNDERLYING SYMBIOSIS BETWEEN ANAEROBES AND WOOD-FEEDING LOWER TERMITES

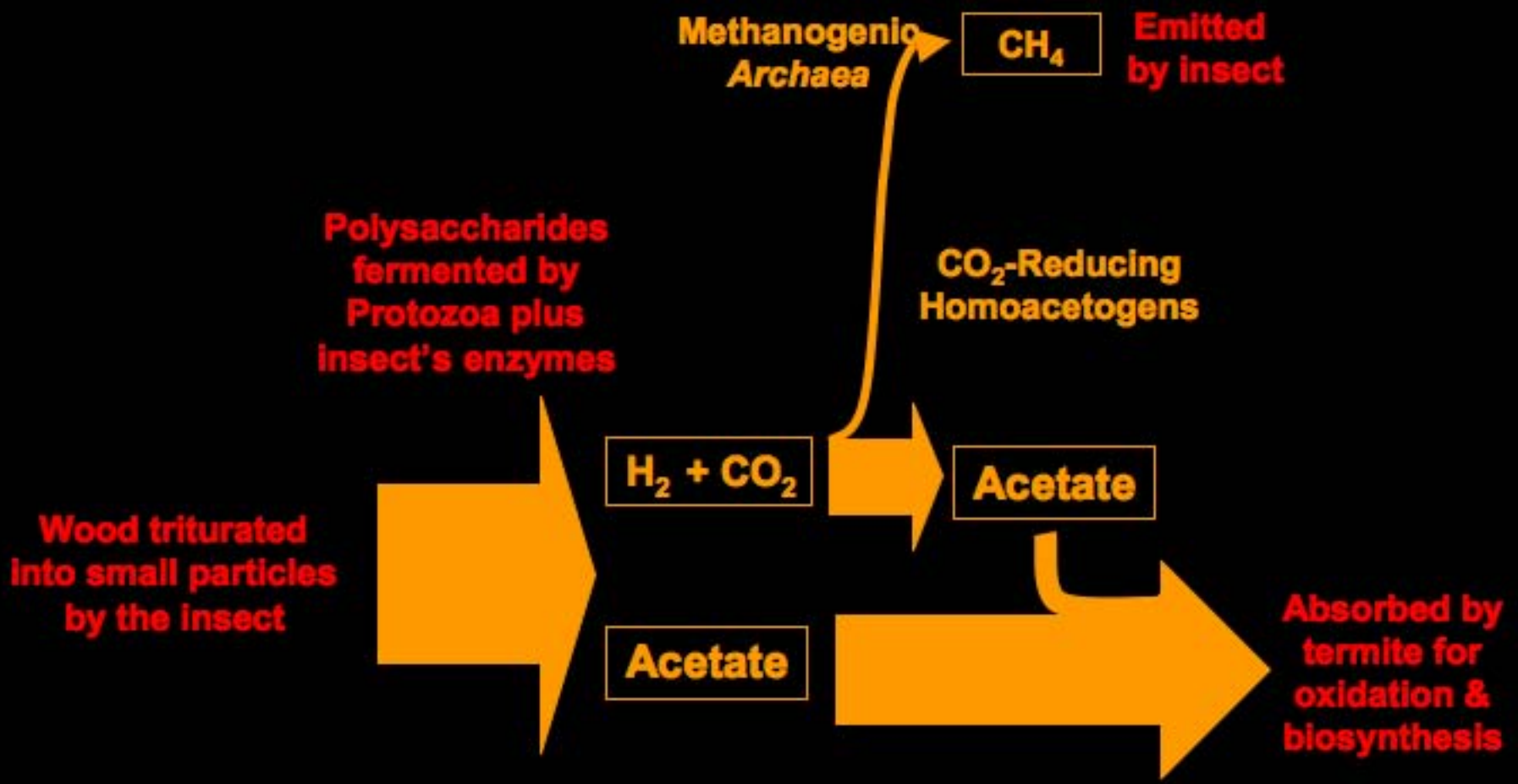


1920 ---- Cleveland • Hungate • Trager • Yamin • Odelson • Breznak ---- Current

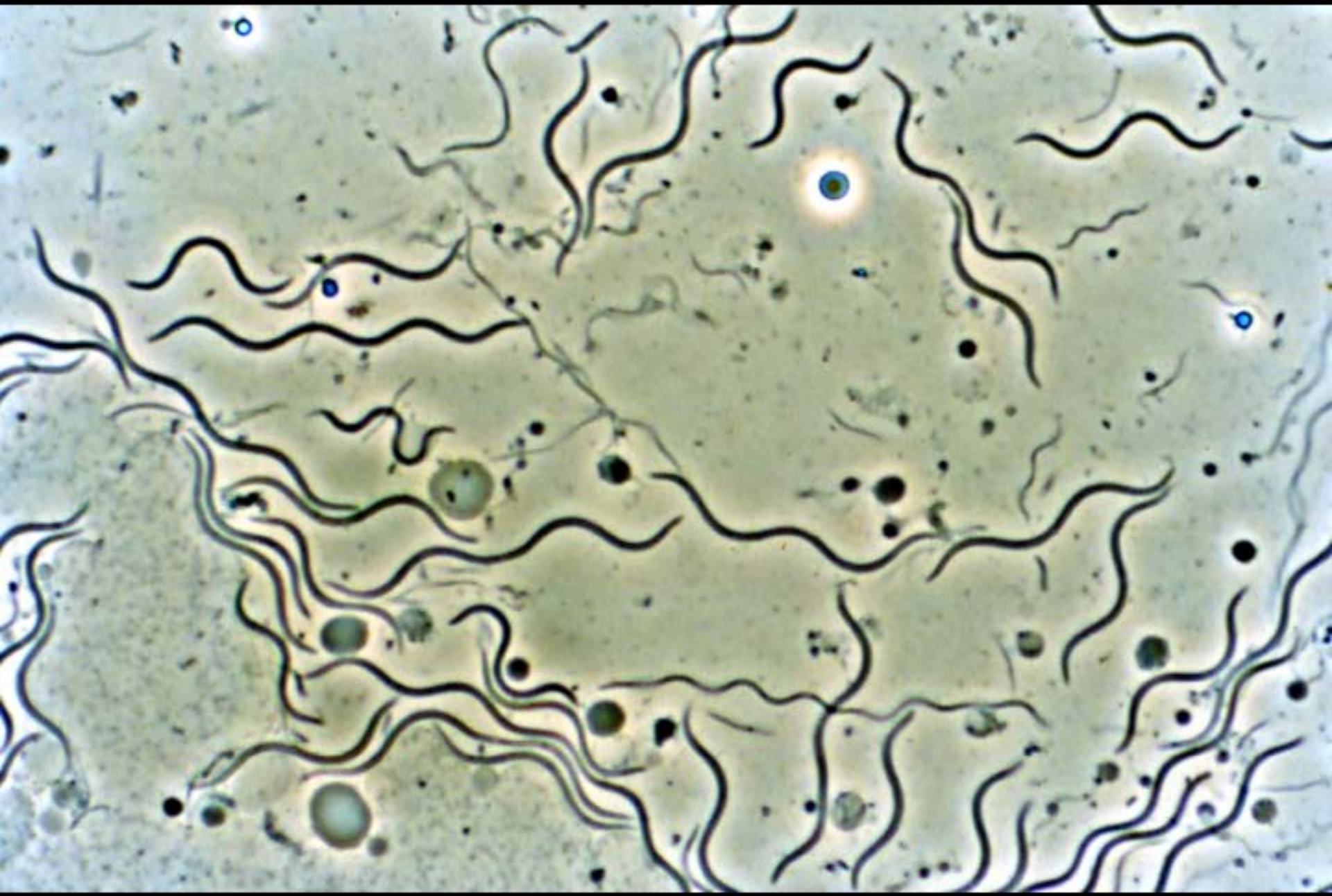


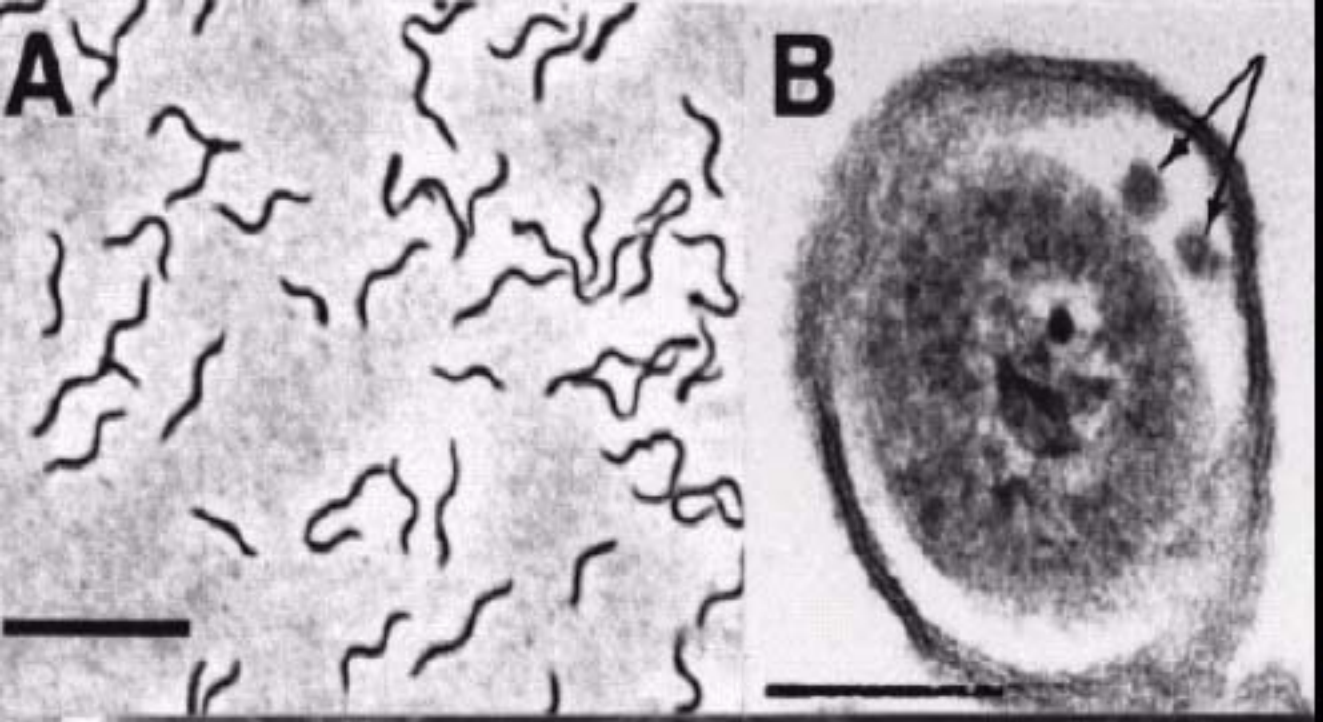
Microbiologists have more fun

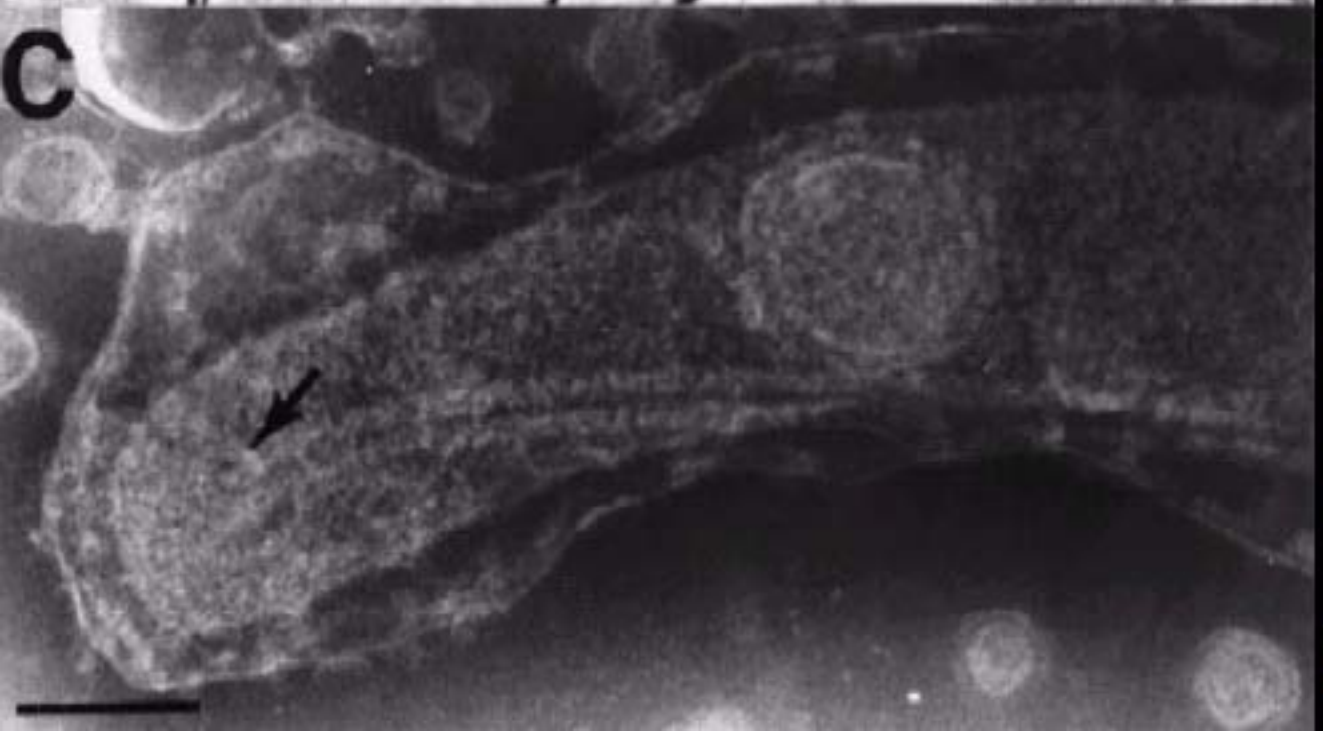
GENERAL SCHEME UNDERLYING SYMBIOSIS BETWEEN ANAEROBES AND WOOD-FEEDING *LOWER* TERMITES



1920 ---- Cleveland • Hungate • Trager • Yamin • Odelson • Breznak • Brune ---- Current







***Treponema primitia* sp. nov.**
Strains ZAS-1 and ZAS-2

H_2 stimulates growth

- Classical H_2:Acetate stoichiometry
- Fix $^{14}CO_2$ into Acetate
- Both C Positions in acetate become labeled with $^{14}CO_2$
- Exhibit CODH and other key activities of Wood-Ljungdahl Pathway

Genomes:
- 3.4 & 3.9 Mb, respectively
- 50% G+C
- compare to 2.8 & 1.1 Mb for *T. denticola* and *T. pallidum*

Leadbetter et al 1999
Graber, Leadbetter & Breznak 2004

Comparing the SARGASSO SEA with the TERMITE HINDGUT

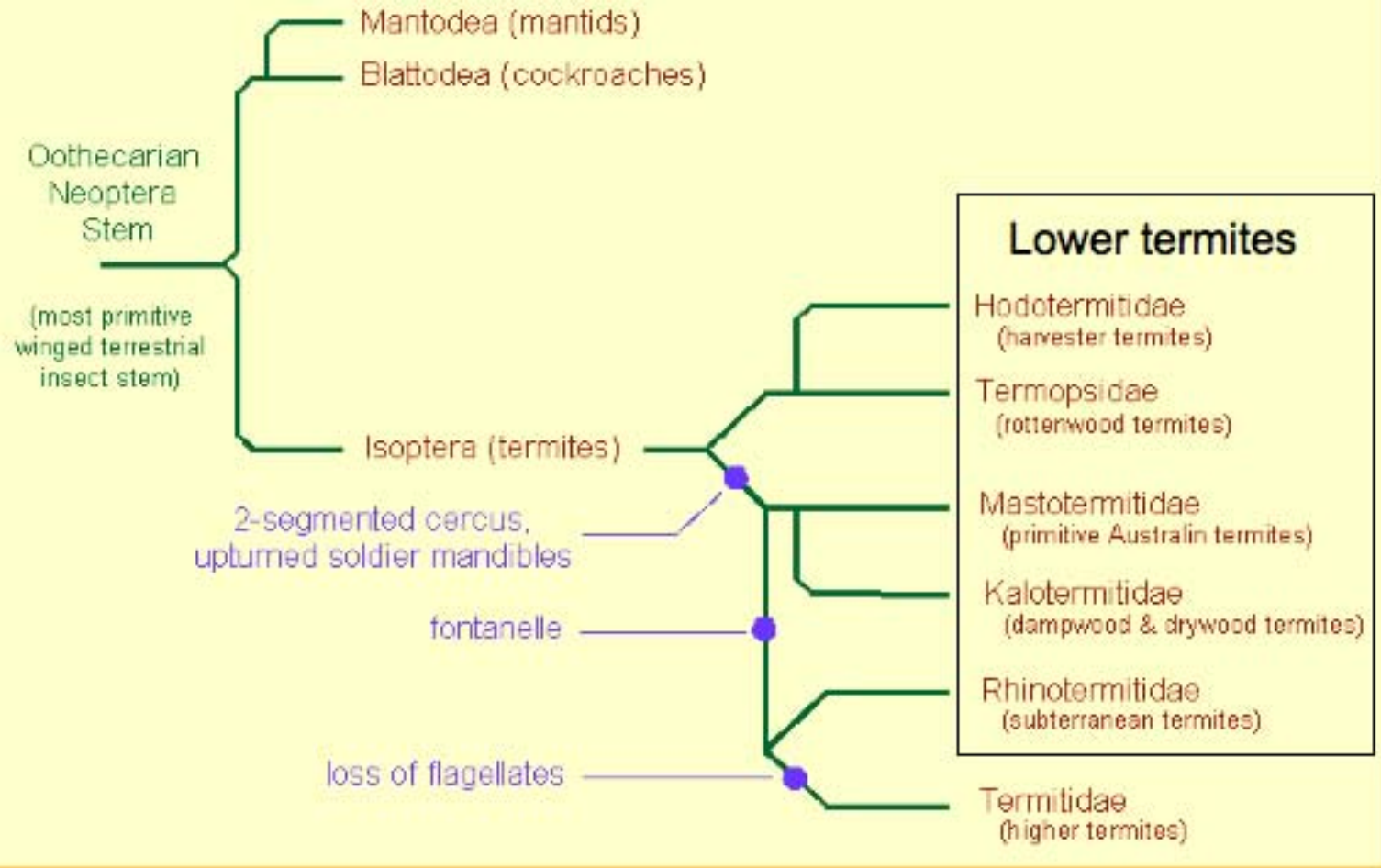
Sargasso Sea - *1,110 x 3,200 km ellipse in the North Atlantic.*

Termite hindgut paunch – *ca. ~1 cubic mm containing ca. 0.5 microliters of fluid, wood particles, and diverse microbes.*

The Sargasso Sea's neuston (surface 1 mm) differs in volume by ca. 19 orders.

The termite hindgut is 5 orders higher in microbial population density.

The hindgut is an example of a tiny-yet-complex, well-bounded ecosystem that is available in large numbers of replicates – *a single nest may contain 500,000 individuals, each with nearly the same exact community dedicated to the metabolism of lignocellulose.*

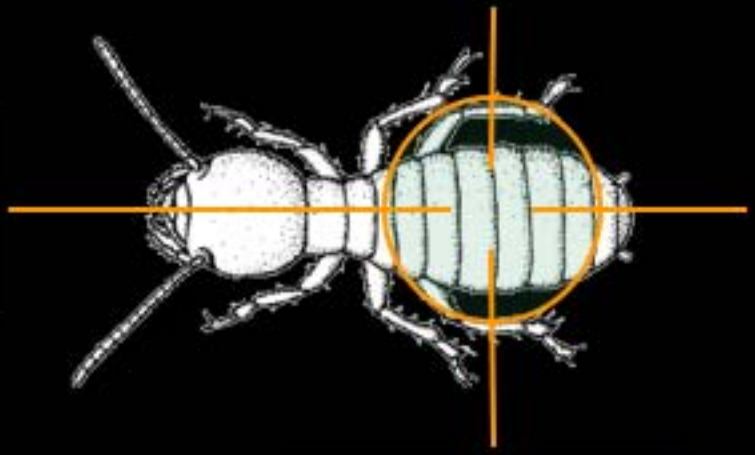


LOWER TERMITES
Contain anaerobic protozoa
Protozoal cellulases known by 1920s
Many bacteria, none well studied

HIGHER TERMITES
NO protozoa
Sources of cellulases no understood
Dominant: biomass abundance
Dominant: termite species richness
Poorly studied despite global reach

Termite Hindgut Microbiota

Community Sequencing Program Team

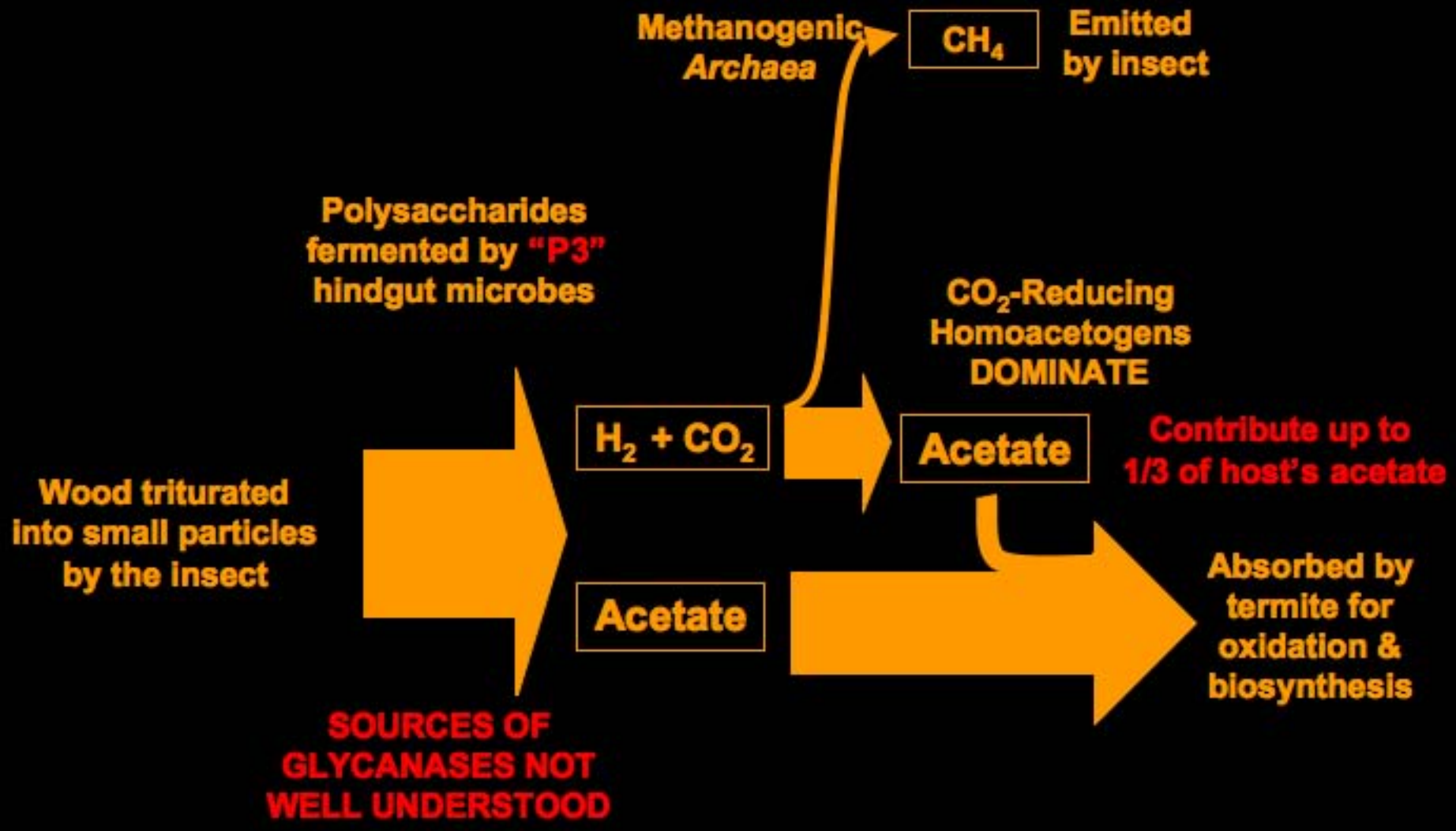


JGI
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

DIVERSA
Evolving the best from nature

INBio
Instituto Nacional
de Biodiversidad

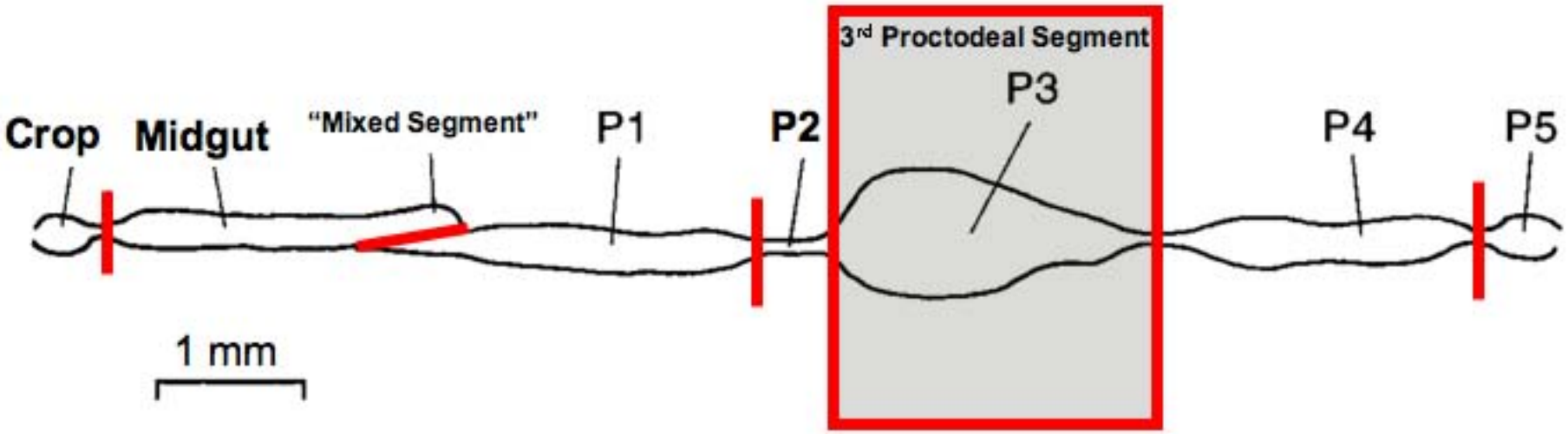
GENERAL SCHEME FOR WHAT LITTLE WE KNEW ABOUT THE ANAEROBIC ACTIVITIES OF WOOD-FEEDING HIGHER TERMITES



COLLECTING *NASUTITERMES* FROM NEST NEAR GRAPILES COSTA RICA

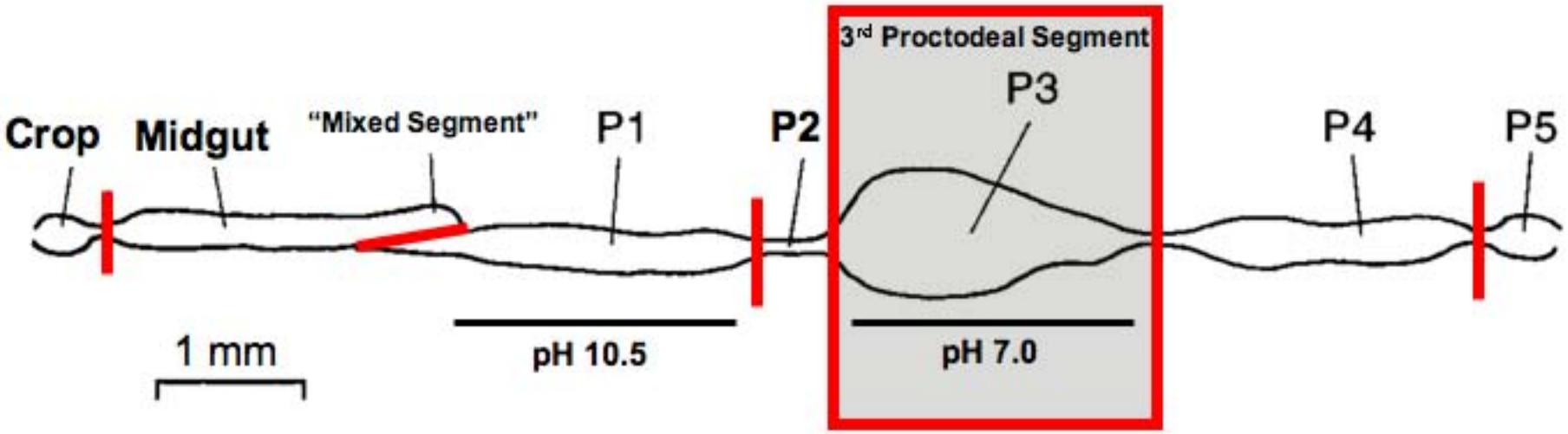

NASUTITERMES WORKERS AND SOLDIERS
Alejandro J. Sánchez Muñoz

SCHEMATIC DIAGRAM OF THE GUT TRACT OF *NASUTITERMES*



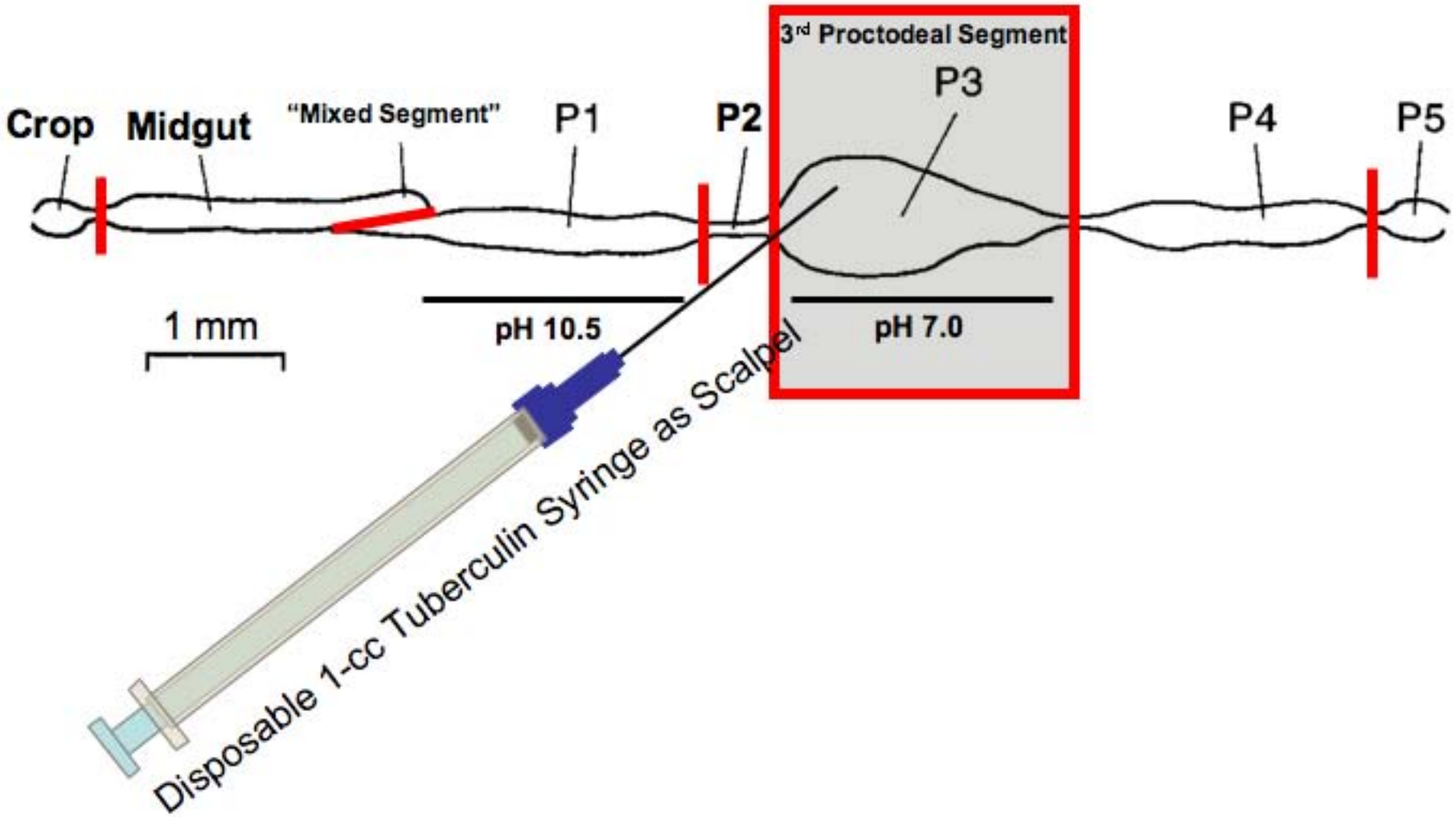
Adapted from Brune Emerson & Breznak 1995

SCHEMATIC DIAGRAM OF THE GUT TRACT OF *NASUTITERMES*



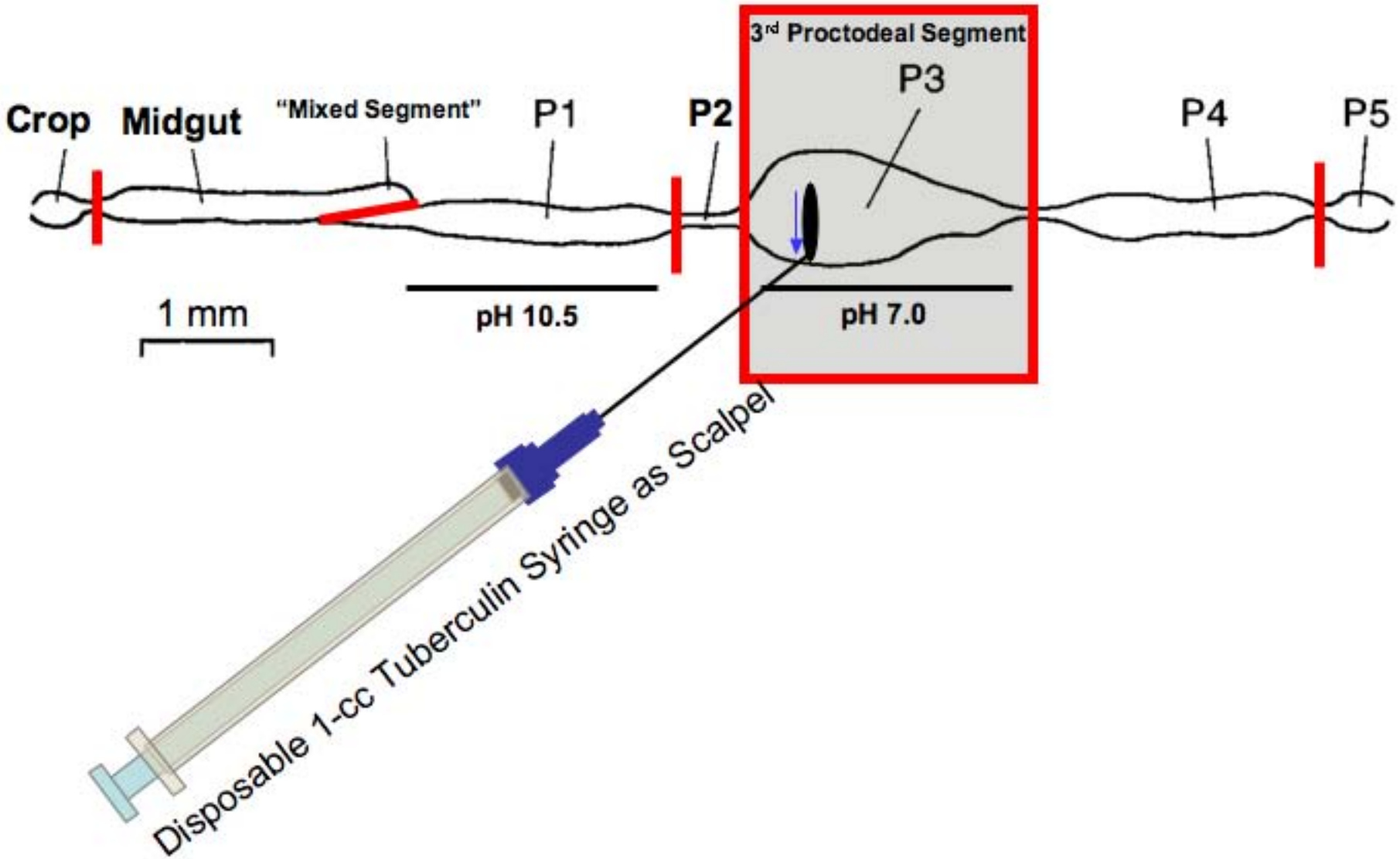
Adapted from Brune Emerson & Breznak 1995

SCHEMATIC DIAGRAM OF THE GUT TRACT OF *NASUTITERMES*



Adapted from Brune Emerson & Breznak 1995

SCHEMATIC DIAGRAM OF THE GUT TRACT OF *NASUTITERMES*



Adapted from Brune Emerson & Breznak 1995

x 200 =

N. CORNIGER P3 LUMINAL CONTENTS *SHOTGUN SEQUENCING*

~61.5 Mb

Phrap assembly:

- **57,600 contigs**
- **largest contig = 13.2 kb**
- **Unassembled singlets = 19,629 reads (29%)**

annotated with fgenesb:

- **82,800 ORFs total**
- **High coding density: 71% of bases were coding**

~ 1% of reads = eukaryotic DNA

This CSP was a pretty risky proposal!

Why?

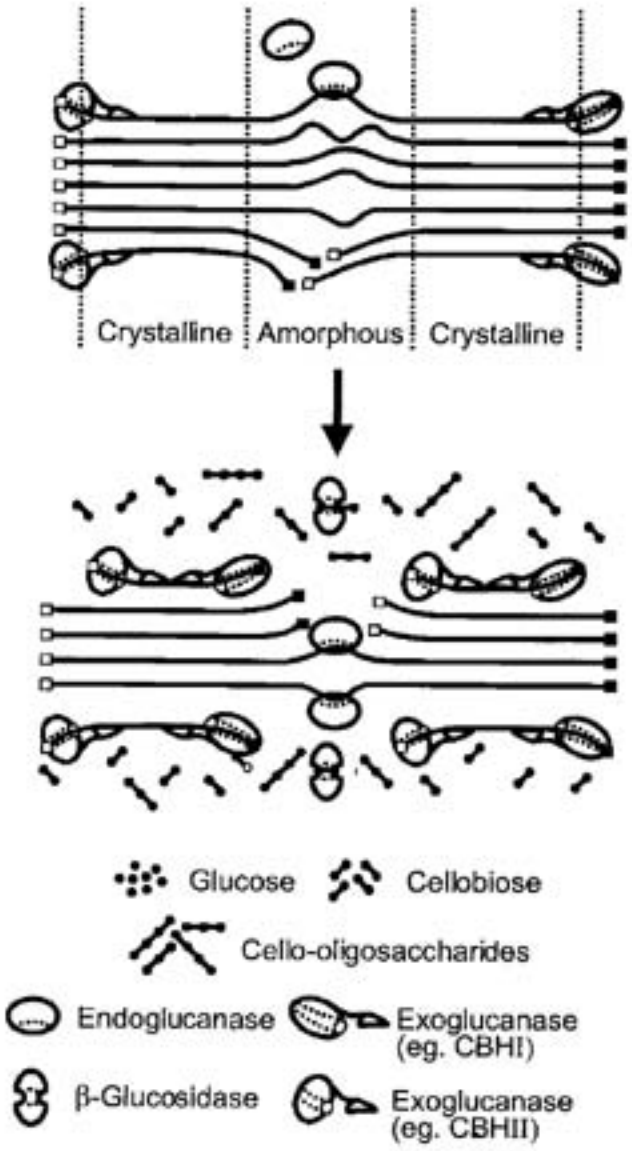
In 150 years of study:

No compelling case had ever been made for a termite gut bacterial involvement in cellulose or xylan hydrolysis.

This had largely been related to the negative results from cultivation studies.

Nevertheless, we were a bit anxious……

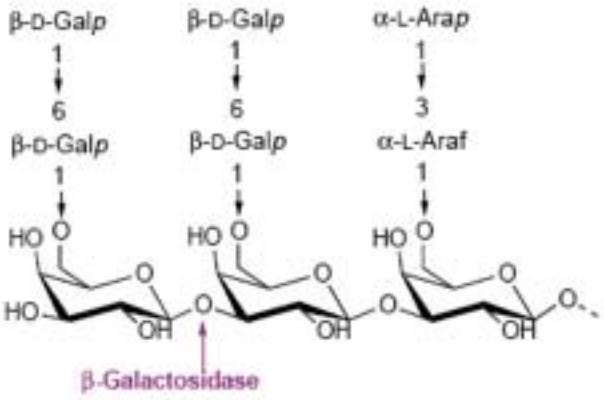
……..Phew!



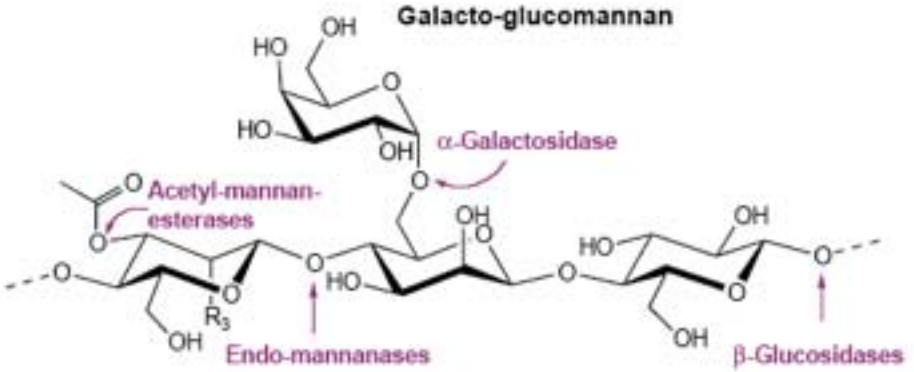
The *Nasutitermes* metagenome encodes at least 200 obvious and diverse endoglucanases and exoglucanases.

MMBR

Arabinogalactan



Galacto-glucomannan

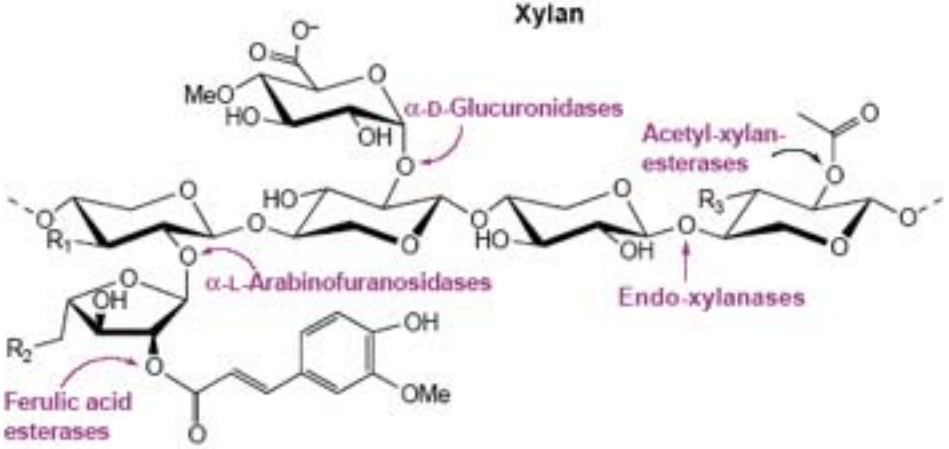


Metagenome encodes:

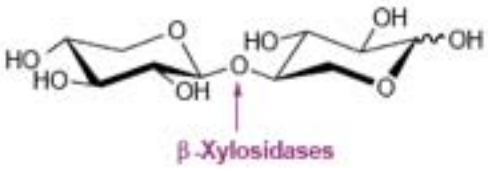
200 xylanases & hemicellulases

500 associated sugar-releasing enzymes

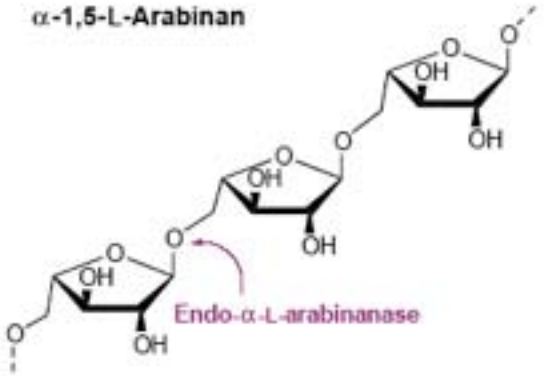
Xylan



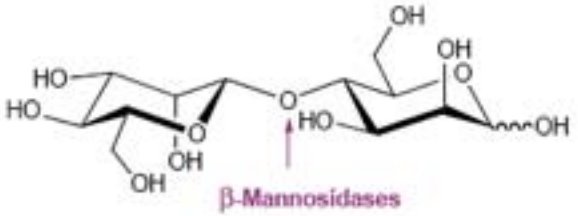
Xylobiose



α-1,5-L-Arabinan

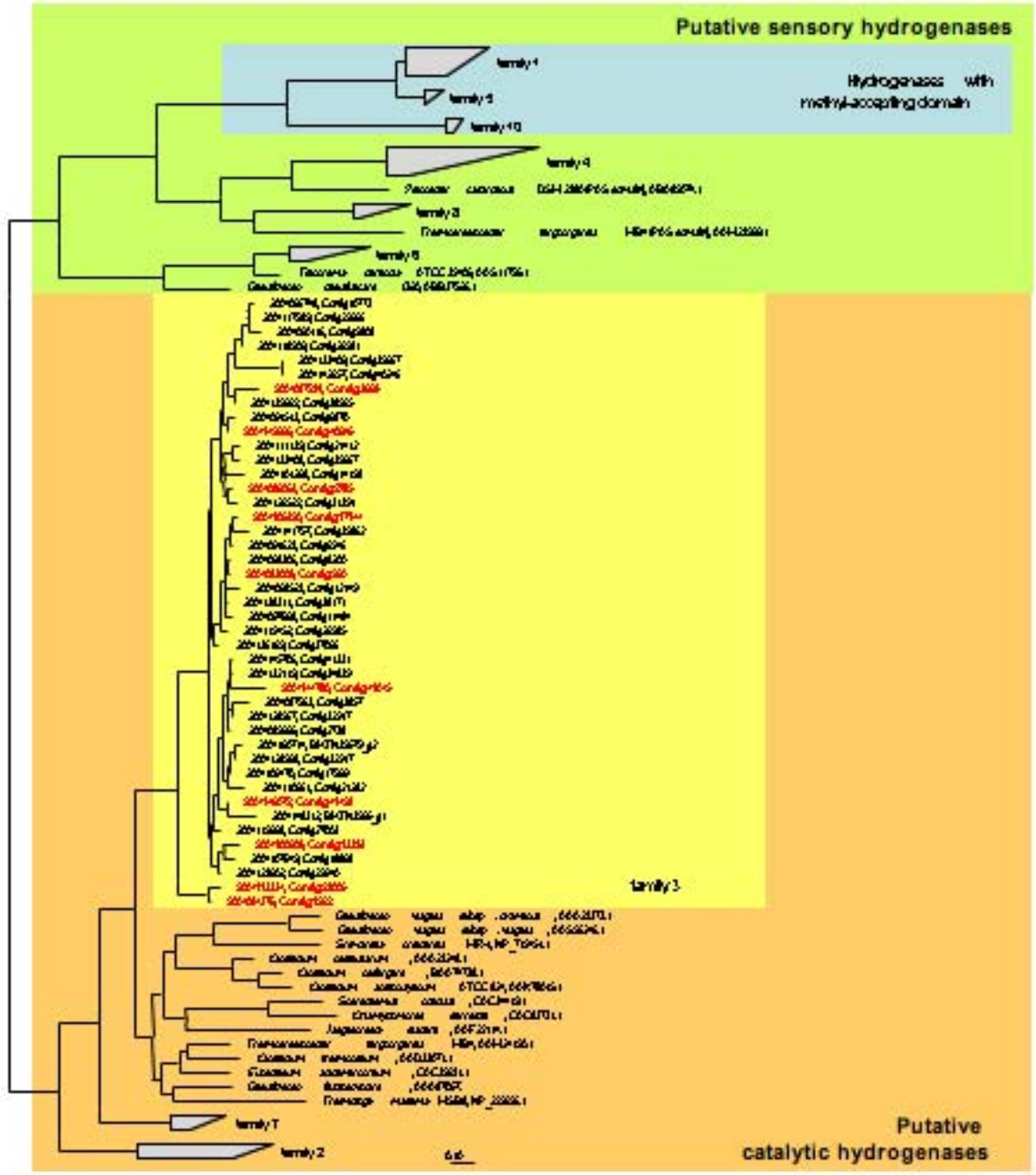


Mannobiose

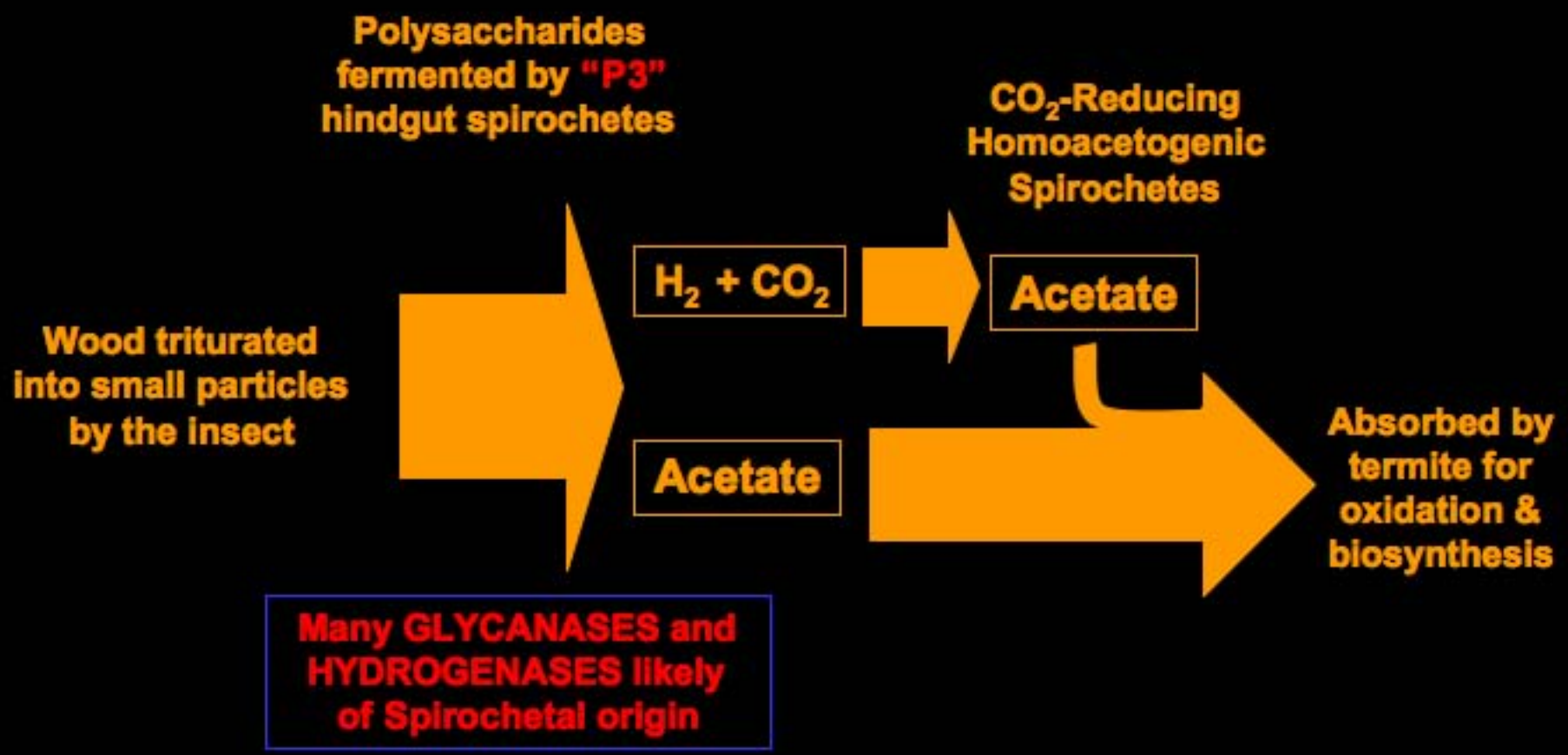


Current Opinion in Microbiology

Phylogenetic diversity of iron-only hydrogenases.



GENERAL SCHEME FOR WHAT WE THINK WE NOW KNOW ABOUT THE ANAEROBIC ACTIVITIES IN THE P3 OF WOOD-FEEDING *HIGHER* TERMITES



Conclusions

- Nasute hindguts are dominated by spirochetes and fibrobacters.
- The gut bacteria encode a rich diversity of wood-degrading enzymes.
- Both cellulose and xylan appear to be extensively targeted.
- Diverse H_2-producing and -consuming processes are present.
- Spirochetes appear to play key roles in all of these activities.

CALIFORNIA INSTITUTE OF TECHNOLOGY 1891

DIVERSA
Evolving the best from nature

INBio
Instituto Nacional de Biodiversidad

JGI
DOE JOINT GENOME INSTITUTE
US DEPARTMENT OF ENERGY
OFFICE OF SCIENCE

Thank you to my "*Termite co-Workers*":

- Liz Ottesen
- Tina Salmassi
- Jian Yuan Thum
- Eric Matson
- Xinning Zhang
- Kasia Gora
- Amy Vu
- Abbie Green
- Nick Ballor
- Alex Romero
- Adler Dillman
- Elaine Hsaio

Funding from NSF Ecosystems Studies &
NSF Microbial Genomes & DOE Genomes to Life